

## **Molecular and morphological evidence for the presence of a new Buthid taxon (Scorpiones: Buthidae) on the Island of Cyprus**

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**Molecular and morphological evidence for the presence of a new Buthid taxon (Scorpiones: Buthidae) on the Island of Cyprus.** - Allozyme data (16 loci) from *Mesobuthus gibbosus* populations in the eastern Mediterranean region show that the Cyprus population is highly distinct, although morphological differentiation is rather weak. This provides evidence for a 'hidden' taxon on Cyprus. This island population is described as a new species.

**Key-words:** Allozymes - Scorpiones - *Mesobuthus* – new species - Cyprus.

### **INTRODUCTION**

Comparison of molecular data from insular and continental populations of a species or of closely related species may provide new insights into the process of speciation with respect to time (Estoup *et al.*, 1996; Baldwin & Sanderson, 1998; Beerli *et al.*, 1996; Gillespie *et al.*, 1998; Hollocher, 1998; Widmer *et al.*, 1998). Island populations are of particular interest for the analysis of colonisation processes (Vachon & Abe, 1988) or for studying founder effect speciation (Templeton, 1980, 1981; Carson & Templeton, 1984; Grant, 1998). *Mesobuthus gibbosus* (Brullé, 1832) (Scorpiones: Buthidae) is widely distributed in the eastern Mediterranean region (Werner, 1928, 1937, 1938; Vachon, 1947a, 1947b, 1948, 1966; Tolunay, 1959; Gruber, 1963, 1966; Kinzelbach, 1975, 1982, 1984, 1985; Vachon & Kinzelbach, 1987; Kritscher, 1993; Crucitti, 1993). Its geographical range includes the mainland (south Balkan, Peloponnesus, Anatolia) and eastern Mediterranean islands (e. g. Cyclades, Sporades, Crete

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and Cyprus). The geographic variation within scorpion species was traditionally examined by using morphological characters like patterns of trichobothria (=‘trichobothriotaxy’) (Vachon, 1974, 1975, 1976; Fet, 1986; Fet & Rechkin, 1989) or variation in the number of pectinal teeth (Kinzelbach, 1975; Michalis & Kattoulas, 1981; Michalis & Dolkeras, 1989). The taxonomic status of different populations of *M. gibbosus* has remained unclear: Kinzelbach (1975) distinguished two subspecies, *M. g. gibbosus* (Brullé, 1832) (south of the Balkan Peninsula, Northern Sporades, Cyclades) and *M. g. anatolicus* (Schenkel, 1947) (Crete, Anatolia, Cyprus). Kritscher (1993) doubted the validity of *M. g. anatolicus* and rejected it.

The widely accepted theory of the salinity crisis (Hsü, 1972; Hsü *et al.*, 1977) states that the Mediterranean sea dried out 5.6 Myrs ago and, consequently, the colonisation of islands via landbridges was possible during a period of 100'000 yrs. Since the refilling of the basin (5.2 Myrs ago) the populations became geographically isolated. Since then, a considerable degree of differentiation between mainland and island populations is expected to have taken place. Alternatively, other genetic population structures than expected are likely if scorpions were introduced by man. Because of several introductions of euscorpiids (Stockwell 1992) caused by man (e. g. Benton, 1991; Goyffon, 1992; Toscano-Gadea, 1998), island populations might not be as isolated as expected.

To clarify the status of some island populations and of the Cyprus population in particular, we carried out a comparative genetic analysis at 16 allozyme loci. Populations from the Peloponnesus, from the south of the Balkans, from Crete, Rhodes, Anatolia and from Cyprus were included. We describe here new morphological characters, which distinguish *Mesobuthus* from Cyprus from the other populations of *Mesobuthus gibbosus* examined.

## MATERIAL & METHODS

*Specimens analysed.* Samples were collected at two sites on the Peloponnesus, GR (Vigla [Arta]; Mathia [Messinia]), two sites on Crete, GR (Vai; Zakros [both in Lassithi]), two sites on Rhodes, GR (Petaloudes; Kolympia), two sites in central Anatolia, TR (Avanos; Hacibectas [both Cappadocia]), one site in southern Anatolia, TR (Selale [Pamphylia]) and two sites on northern Cyprus (Tepebasi; Kantara [both Turkish part]). The scorpions were transported alive to the laboratory, killed by deep-freezing and stored at -80°C prior to electrophoresis. For the morphological examination, the specimens were later transferred into 75% ethanol. *Androctonus mauretanicus* (Pocock, 1902) from Agadir (MA) and *Androctonus crassicauda* (Olivier, 1807) from Urfa (TR) were used as outgroup species for phylogenetic analyses. The sampling sites are shown in Fig. 1.

*Allozyme analysis.* Horizontal starch gel electrophoresis of allozymes was carried out according to the protocols described in Gantenbein *et al.* (1998) and Murphy *et al.* (1996). We scored 16 loci on three buffer systems: N-(3-Aminopropyl)-morpholine-citrate (AC, pH 6.2, modified from Clayton & Tretiak, 1972), Tris-citrate (TC, pH 7.3, Ayala *et al.*, 1972) and Tris-borate-EDTA (TBE, pH 9.3, modified from Ayala *et*

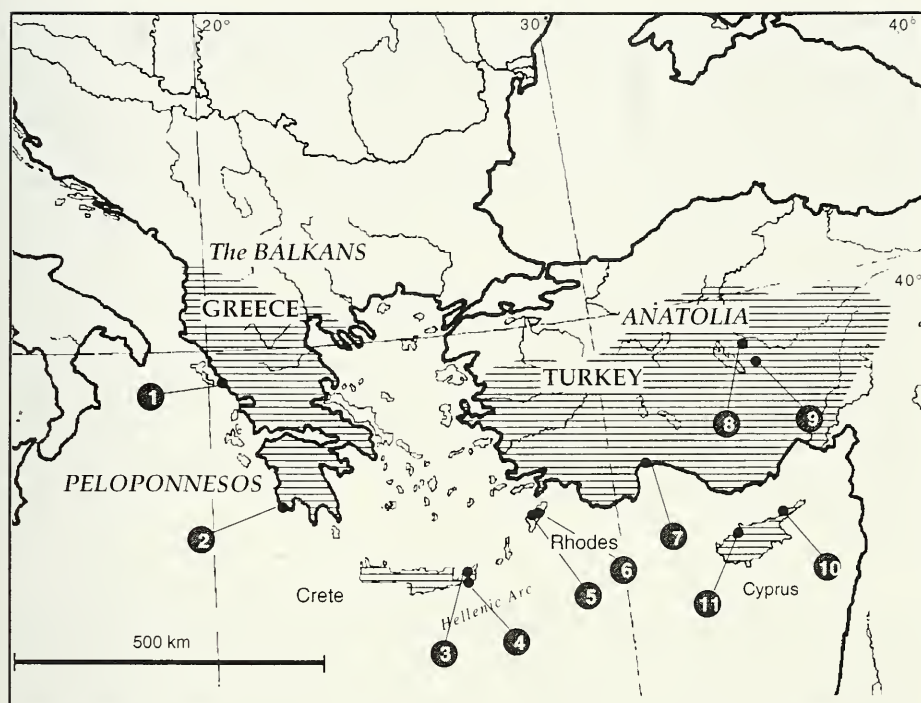


FIG. 1

Sampling sites of the analysed *Mesobuthus* populations: 1: Vigla, GR, 2: Mathia, Gr, 3: Vai, GR, 4: Zakros, GR, 5: Kolympia, GR, 6: Petaloudes, GR, 7: Selale, TR (3 sites), 8: Hacibectas, TR, 9: Avanos, TR, 10: Kantara, TR, 11: Tepebasi, TR.

*al.*, 1972). The loci scored were: ALPDH (Alanopine dehydrogenase; EC 1.5.1.17), ARK (arginine kinase; EC 2.7.3.3), AAT-1 and AAT-2 (aspartate aminotransferase; EC 2.6.1.1), DDH (dihydrolipoamide oxidase; EC 1.8.1.4), PGI (GPI) (glucose-6-phosphate isomerase; EC 5.3.1.9), GTDH (glutamate dehydrogenase; EC 1.4.1.2), IDH-1 and IDH-2 (isocitrate dehydrogenase; EC 1.1.1.42), MDH-1 and MDH-2 (malate dehydrogenase; EC 1.1.1.37), MPI (mannose-6-phosphate isomerase; EC 5.3.1.8), PGM (phosphoglucomutase; EC 5.4.2.2), 6-PGD (6-phosphogluconate dehydrogenase; EC 1.1.1.44), PK (pyruvate kinase; EC 2.7.1.40), and SOD (superoxide dismutase; EC 1.15.1.1). We refer to the observed electromorphs as alleles which are identified by their electrophoretic mobility relative to the most common mobility in the *Euscorpis flavicaudis* (de Geer, 1778) population from Lauris, France (assigned mobility=100) as described in Gantenbein *et al.* (1998). To assess the genetic variability within each population, the mean number of alleles per locus, the percentage of polymorphic loci and the mean heterozygosity were calculated by the direct count method and by Nei's (1978) unbiased estimate. Nei's genetic distance (1972) was calculated from pairwise

comparisons of populations using the program GENDIST from the PHYLIP 3.5 package (Felsenstein, 1995). Nei's distance is expected to rise linearly with time since the complete separation of gene pools, if sufficiently large numbers of loci are measured (Felsenstein 1984; Nei 1987). Using Nei's pairwise distances as an input matrix, an additive tree was created by the neighbour-joining algorithm (NJ) (Saitou & Nei, 1987). NJ is assumed to be a good heuristic approach for estimating the minimum evolution tree (Page & Holmes, 1998) and allows for different rates among lineages in contrast to the UPGMA clustering algorithm. Alternatively, an unrooted maximum likelihood tree was calculated using the computer program CONTML. It estimates phylogenies by the restricted maximum likelihood (REML) method, based on the Brownian motion model (Cavalli-Sforza & Edwards, 1967). The REML algorithm was formerly described in Felsenstein (1973, 1981) and uses less parameters than the full ML analysis and is, therefore, assumed to be more consistent. Additionally, the program calculates branch lengths and rough confidence intervals for the branches. Bootstrap values were obtained based on 1000 pseudo-replicates of allele frequencies using the SEQBOOT routine in PHYLIP.

## RESULTS

*Genetic variability.* In general, the observed genetic variability was rather low within the *Mesobuthus* samples. The mean observed heterozygosity was  $0.07 \pm 0.05$ , one to six out of 16 loci were polymorphic at the 0.95 criterion. The mean number of alleles ranged from 1.0 to 1.5 per locus. All analysed populations were fixed at many loci (Table 1). However, this variation was not evenly distributed among *Mesobuthus* samples. At five loci, more than two electromorphs were detected, whereas all populations were fixed for the same allele at three loci (Idh-2, Mdh-1, Pk). The Cyprus samples were fixed for private alleles (alleles that were not found in any other population) or showed polymorphisms with private alleles at six loci (Aat-1, Aat-2, Alpdh, Ldh-2, Mdh-2, 6-Pgd) in addition to minor differences in allele frequencies at the locus Mpi (Table 1). On the other hand, if compared to the mainland populations, the island populations from Crete and Rhodes showed private alleles at one locus only (samples Zakros and Vai at Aat-1 and samples Kolympia and Petaloudes at 6-Pgd, respectively). The island samples and the mainland samples differed mainly in the allele frequencies.

The outgroup comparison with *A. mauretanicus* and *A. crassicauda*, respectively, revealed alleles at eight loci that were not found in *Mesobuthus*, whereas, with respect to allele frequencies, the two *Androctonus* species differed from each other only at two loci.

TABLE 1

Allele frequencies and sample sizes of eleven *Mesobuthus* populations. *Androctonus mauretanicus* (MA) and *A. crassicauda* (TR) were used as outgroup species. Alleles were labelled as described in the material & methods section. Measures of genetic variability for each population are given at the bottom of the table. Private alleles of the Cyprus samples and the heterozygosity



Genus/species Region		<i>M. gibbosus</i>									<i>M. cyprius</i>		<i>Androctonus</i>	
		Greek mainland		Crete	Rhodes		S. Ana- tolia	C. Ana- tolia		Cyprus	Morocco	Turkey		
Site		Vigla	Mathia	Zakros	Vai	Kolym- bia	Peta- loudes	Selale	Avanos	Haci- bectas	Tepe- basi	Kan- tara	Aourir	Urfa
Sample size	Allele	(2)	(15)	(5)	(2)	(6)	(6)	(16)	(5)	(3)	(20)	(4)	(14)	(6)
Aat-1	111 104 94 82 80 75	1.00	1.00			1.00	1.00	1.00	1.00	1.00	0.30 0.70		1.00	0.08 0.75 0.17
				1.00	1.00						0.35 0.65	0.63 0.24		
Aat-2	117 110 109 106 100	1.00	0.97	1.00	1.00	1.00	1.00 0.09	0.91	1.00	1.00	0.65	0.13 0.24	1.00	1.00
Alpdh	105 100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ark	102 98	1.00	1.00	1.00	1.00	0.67 0.33	0.17 0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ddh	99 98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.05 0.95	1.00	1.00	1.00
Gtdh	100 96	1.00	1.00	1.00	1.00	0.67 0.33	0.25 0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Idh-1	104 98 96 93 89	1.00	1.00	1.00	1.00	1.00	0.08 0.92	0.81	0.90 0.10	1.00	0.97	1.00	1.00	1.00
								0.19			0.03			
Idh-2	93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-1	105 104	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-2	92 82 72	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
													1.00	0.08 0.92
Mpi	160 154 152 150 145 142 135 130 110	0.25 0.25	1.00	0.50 0.50	1.00	1.00	1.00	0.97 0.03	0.90 0.10	1.00	0.62	1.00		
											0.38		0.11	0.75 0.25
		0.50												0.57 0.25 0.18
6-Pgd	112 111 104 100 93 82					0.08	0.08						1.00	1.00
		1.00	1.00	1.00	1.00	0.92	0.92	1.00	1.00	1.00	0.03			
											0.94 0.03	0.12 0.88		
Pgi (GPI)	95 87	0.25 0.75	1.00	1.00	1.00	0.42 0.58	0.58 0.42	0.06 0.94	1.00	1.00	0.08 0.92	1.00	0.04 0.96	0.67 0.33
Pgm	88 81	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pk	100 98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod	110 109 103			1.00	1.00	0.67	0.25	1.00	1.00	1.00			1.00	1.00
		1.00	1.00			0.33	0.75				1.00	1.00		
Mean no. of alleles per locus		1.2 (0.1)	1.1 (0.1)	1.1 (0.0)	1.0 (0.1)	1.3 (0.1)	1.4 (0.1)	1.3 (0.1)	1.1 (0.1)	1.0 (0.0)	1.5 (0.2)	1.2 (0.1)	1.2 (0.1)	1.3 (0.2)
Percentage of polymorphic loci		12.5	0.0	6.3	0.0	31.3	37.5	18.8	12.5	0.0	37.5	12.5	6.3	25
Heterozygosity (observed)		0.06 (0.0)	0.0 (0.0)	0.01 (0.0)	0.0 (0.0)	0.17 (0.1)	0.14 (0.1)	0.04 (0.0)	0.03 (0.0)	0.0 (0.0)	0.08 (0.0)	0.06 (0.0)	0.03 (0.0)	0.09 (0.1)
Heterozygosity (expected)		0.08 (0.1)	0.0 (0.0)	0.04 (0.0)	0.0 (0.0)	0.13 (0.1)	0.12 (0.1)	0.04 (0.0)	0.03 (0.0)	0.0 (0.0)	0.11 (0.1)	0.05 (0.0)	0.04 (0.0)	0.09 (0.1)

TABLE 2

Calculated pairwise distance matrix (Nei's D 1972) for all 13 samples of *Mesobuthus*. In bold the genetic distance values of pairwise comparisons that involved one of the two Cyprus samples.

Region Population	Greek mainland Vigla Mathia		Crete Zakros Vai		Rhodes Kolympia Petaloudes		Anatolia Selale Avanos		Hacibectas	Cyprus Tepebasi Kantara		Outgroup Aourir
Vigla												
Mathia	0.03											
Zakros	0.42	0.41										
Vai	0.44	0.47	0.02									
Kolympia	0.13	0.10	0.21	0.26								
Petaloudes	0.05	0.03	0.35	0.42	0.05							
Selale	0.45	0.39	0.16	0.21	0.19	0.33						
Avanos	0.34	0.29	0.23	0.28	0.11	0.24	0.07					
Hacibectas	0.34	0.29	0.23	0.29	0.10	0.23	0.07	0.00				
Tepebasi	<b>0.31</b>	<b>0.28</b>	<b>0.78</b>	<b>0.84</b>	<b>0.44</b>	<b>0.33</b>	<b>0.87</b>	<b>0.70</b>	<b>0.69</b>			
Kantara	<b>0.39</b>	<b>0.33</b>	<b>0.82</b>	<b>0.92</b>	<b>0.50</b>	<b>0.39</b>	<b>0.95</b>	<b>0.78</b>	<b>0.77</b>	0.03		
Aourir	1.40	1.37	1.13	1.15	1.09	1.32	0.95	0.80	0.81	1.32	1.35	
Urfa	1.49	1.55	1.26	1.28	1.11	1.30	1.04	0.88	0.90	1.44	1.46	0.14

*Genetic differentiation and phylogenetic analyses.* The calculated Nei's distances from pairwise comparisons of populations within *Mesobuthus* ranged from 0.00 to 0.95 (Table 2). The distance values between the two outgroup species and *Mesobuthus* were at least 0.80 and ranged up to 1.55. If the two samples from Cyprus are compared with the other *Mesobuthus* samples, it is obvious that the Cyprus populations are considerably distinct from all others, i. e. Vigla, Mathia, Kolympia, Petaloudes, Zakros, Vai (all GR), Selale, Avanos, Hacibectas (all TR), by rather high distance values (0.31 - 0.95).

The topologies of the NJ tree and of the maximum likelihood (ML) tree (-ln likelihood 544.123; 4909 trees examined) are generally congruent and differ from each other only in the position of the Cretean clade (Figs 2-3). This clade clusters separately in the NJ tree (Fig. 2) and groups with the sample of Selale (south Anatolia) in the ML tree (Fig. 3). However, both approaches revealed four main clades within *Mesobuthus* (Figs 2-3). The first clade is composed of the samples from the mainland of Greece (Vigla and Mathia) and island Rhodes (Petaloudes and Kolympia) and is found at a relatively low distance value. The two samples from Crete are found in a second independent clade, and the populations from Anatolia as a third clade. However, the Cyprus clade remains clearly separated from all other clades by a rather high tree length in both trees, and is confirmed as an offshot from the mainland. The bootstrap analysis supports four nodes of the trees with very high values (>90%), these are observed for the clades grouping the Cyprus samples (Tepebasi and Kantara), the central Anatolian samples (Avanos and Hacibectas), the Crete samples (Zakros and Vai), and the *Androctonus* species, respectively. The node between the Peloponnesian clade and the Cyprus clade is well supported (60% and 77%, respectively). *Androctonus* is confirmed as outgroup by a bootstrap value of 99%. However, the other nodes are weakly supported. The weakest support is found for the clade that contains the Cretean and Anatolian samples. Noteworthy, the outgroup species *A. mauretanicus* and *A. crassicauda* are separated by about the same genetic distance that is found among the samples

of the Peloponnesus / Rhodes clade and the Anatolian clade, although the outgroup samples are treated as different species and are geographically separated by a large distance (approximately 3000 km).

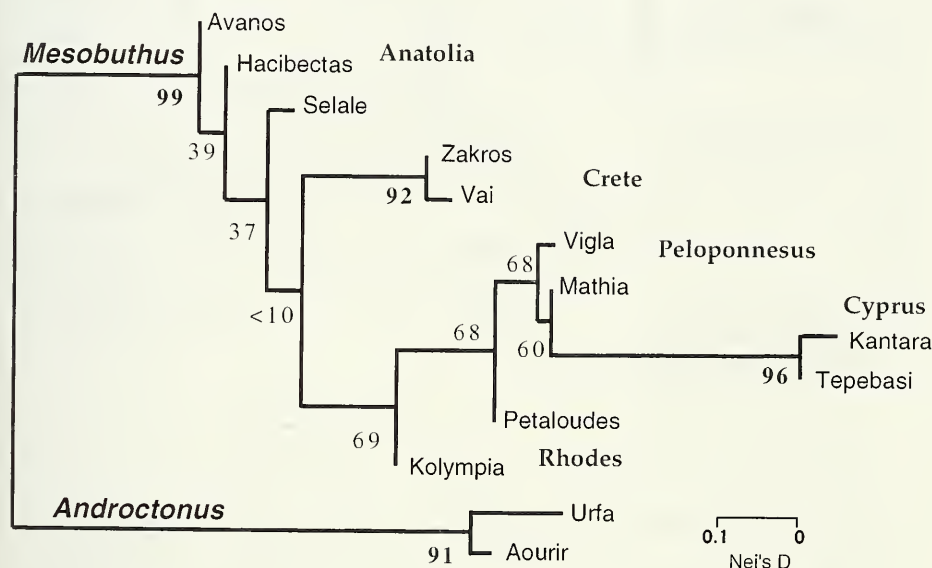


FIG. 2

NJ tree based on Nei's distance relating island and mainland populations of *Mesobuthus* inferred from 16 allozyme loci. *A. crassicauda* from Urfa (TR) and *A. mauretanicus* from Aourir (MA) were used as outgroups.

## TAXONOMY

### *Mesobuthus cyprius* Gantenbein & Kropf, sp. n.

Figs 4-28; Tab. 1

**Type material:** Holotype: 1 male, Tepebasi, Northern Cyprus, 26. V. 1998, Natural History Museum Berne, Switzerland (NMBE). Paratypes (collected at the type locality): 1 male, 20. V. 1998, 1 female, 26. V. 1998, NMBE. 1 male, 20. V. 1998, 1 female, 26. V. 1998, Natural History Museum Basel, Switzerland; all specimens leg. A. Scholl (Berne, CH).

**Other material examined:** Morphology: *Mesobuthus cyprius* sp. n.: 6 males, 11 females from Tepebasi, 1 male, 1 female from Kantara, Cyprus. For a comparison, also specimens of *Mesobuthus gibbosus* from Rhodes, GR (31 specimens), Peloponnesus, GR (7), Crete, GR (20), Euboea, GR (1), Selale, TR (12) and central Anatolia, TR (3) were investigated.

**Allozyme study:** *Mesobuthus cyprius* sp. n., *Mesobuthus gibbosus*, *Androctonus mauretanicus*, *Androctonus crassicauda*: See Table 1.

**Diagnosis:** *Mesobuthus cyprius* sp. n. can be distinguished unambiguously only by the shape of the basal lobes of the hemispermatophores. These are slender and acutely pointed teeth in *Mesobuthus cyprius* sp. n. (Figs 8-9), while in all populations of *M. gibbosus* examined they form scales with more or less distinct blunt tips (Figs 29-

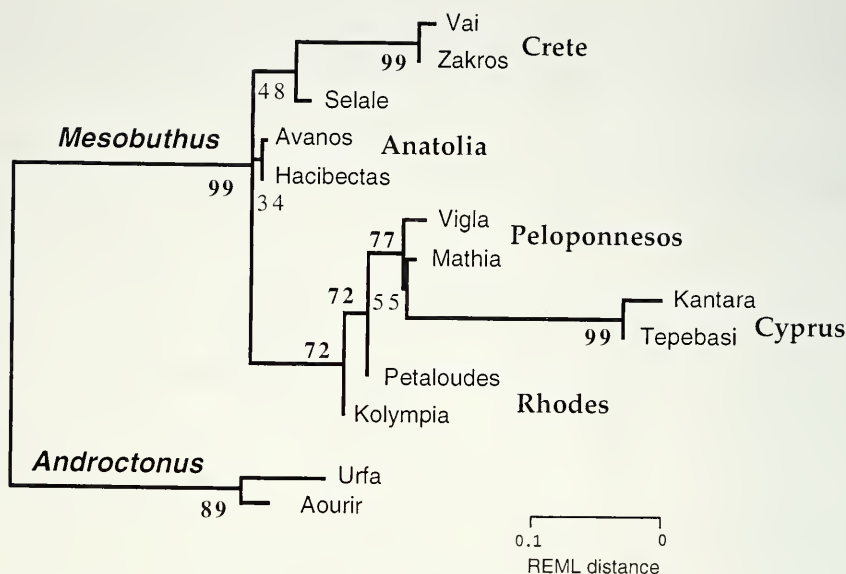


FIG. 3

Rooted maximum likelihood (ML) tree of island and mainland populations of *Mesobuthus*.

33; Vachon, 1948: fig. 1). Moreover, the hemispermaphore of the new species is considerably smaller than in *M. gibbosus*.

**Description:** *Measurements* (in mm): No apparent size dimorphism, although females longer, and males with a longer metasoma. Total length (measured dorsally from anterior margin of carapace to tip of stinger, with telson in horizontal position): Males 45-55, females 45-60. Carapace length (measured dorsally along midline): males 5.0-5.7, females 5.0-6.0. Carapace width (maximum distance between postero-lateral edges): males 5.5-6.0, females 5.5-6.6. Metasoma length: males 28-37, females 27-35 (measured dorsally from anterior margin of first segment to tip of stinger).

*Carapace* (Figs 4, 5): trapezoid-shaped, colour pattern more or less distinct, especially lateral pigmented areas hardly visible in some specimens. Granulation: anterior median, central median and central lateral carinae distinct, granulae in remaining areas mostly weak. Anterior margin with transverse row of bristles.

*Mesosoma* (Figs 5, 6): Tergites I-VI with a pattern of pigmented longitudinal stripes, i.e. one median stripe and two pairs of lateral stripes (one paramedian, one ectal pair). These stripes partly broken, especially on tergite 6, colour varying from dark grey to medium brown (only alcohol preserved material examined, colour pattern more distinct in juveniles than in adults, in some adults hardly visible). Interspace between median and paramedian stripe less than 1-1.5 times as wide as paramedian stripe at posterior margin of tergites IV-VI. Tergite VII with more or less pronounced median



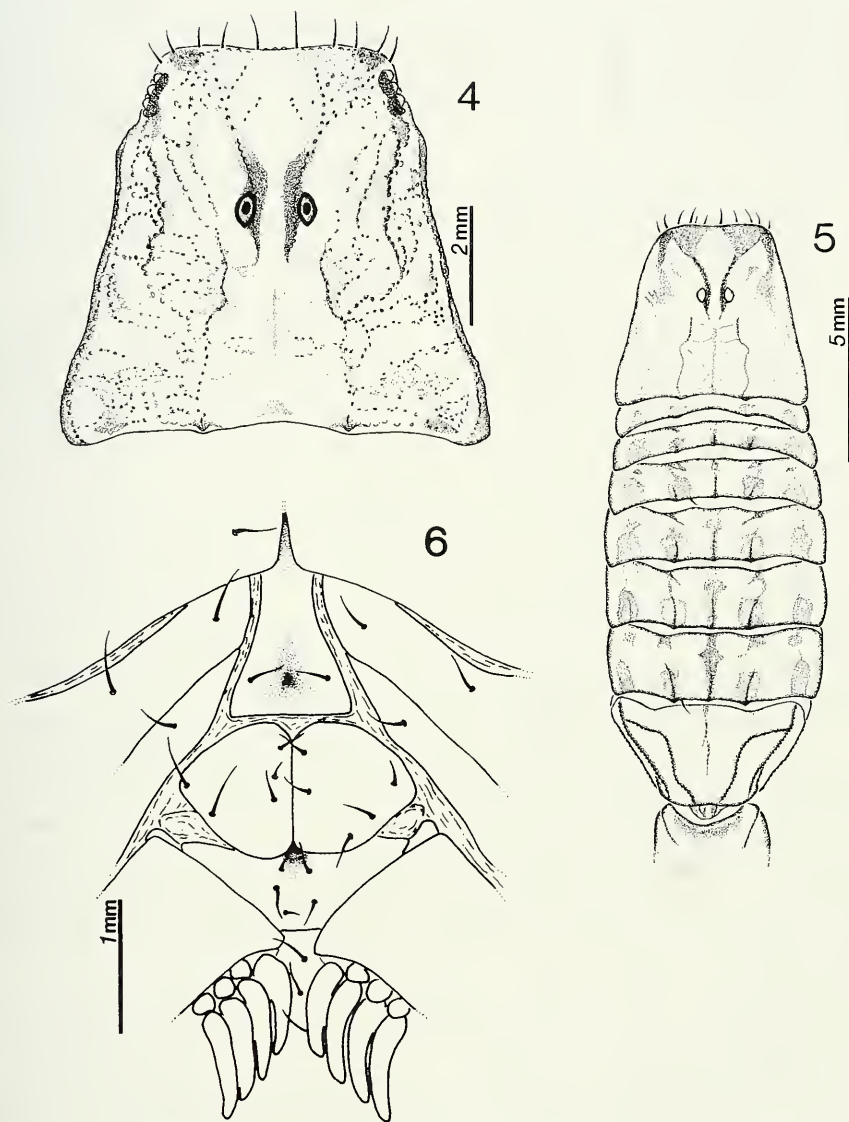


FIG. 4-6

*Mesobuthus cyprius* sp. n. – 4. Female carapace, dorsal view. – 5. Male carapace and mesosoma, dorsal view. – 6. Sternum and genital opercula, ventral view.

keel bearing granula. Tergites only sporadically with bristles or without. Sternites smooth, at least some of them with white guanin spots; these spots sometimes distributed all over the ventral and occasionally also the dorsal surface. Sternum subtriangular, with deep median depression flanked by a pair of bristles (Fig. 6). Genital opercula with a few scattered bristles (Fig. 6). Pectines with 20-23 (females) or 26-30 (males) teeth.

*Hemispermatoaphore* (Figs 7-9): Long, slender, of the flagelliform type. Four lobes at truncal flexure: internal lobe biggest, with a small distal hook, close to the external lobe, partly embracing it. Basal lobe developed as a slender and acutely pointed tooth.

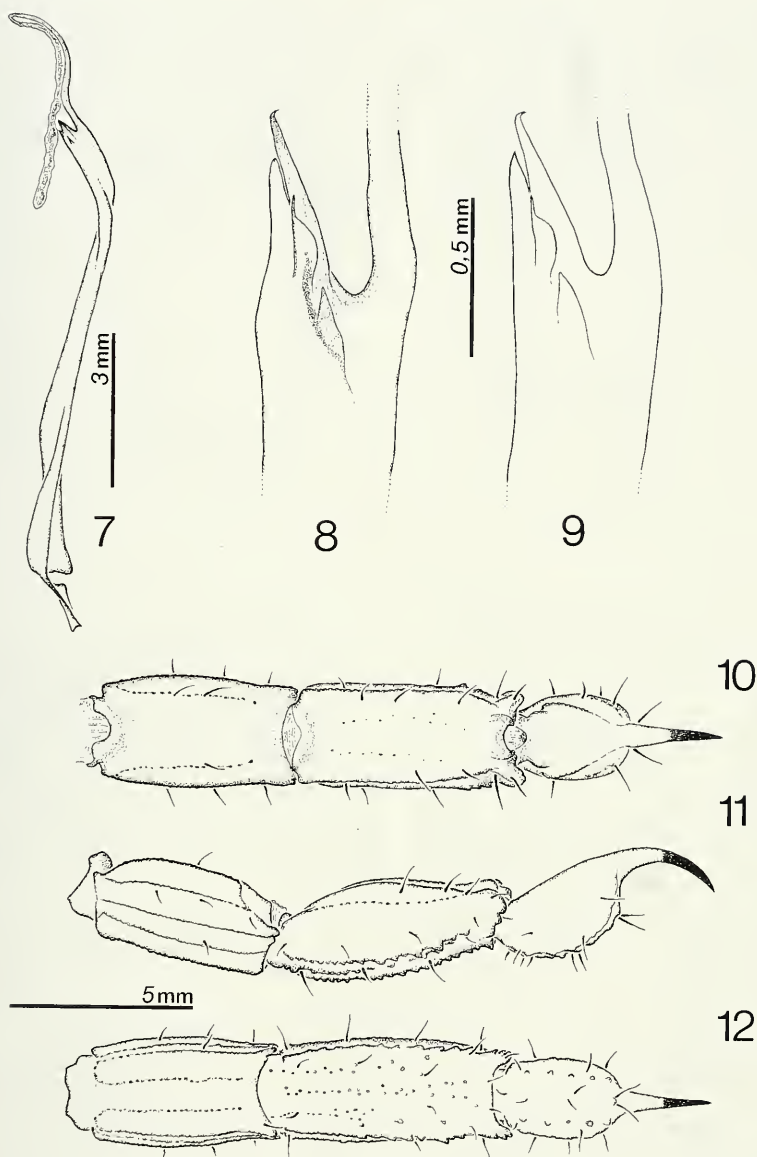
*Metasoma* (Figs 10-12): Segments I-IV with 10 longitudinal keels formed by rows of tubercles; one additional pair of keels mediadorsally being indistinct and hardly visible (not illustrated). Fourth segment 1.79 – 2.44 times longer than high in males, 1.54 – 1.58 times in females (length measured dorsally, height measured as distance between dorsal and ventral keel). Segment V granulate ventrally, with 3-4 blunt teeth laterally at distal edge. Telson with dark tip of stinger, granulate on ventral side, with several short bristles, one ventral pair of them rising from subaculeate tubercle.

*Chelicera* (Figs 13-14): Tibia (nomenclature following Hjelle 1990) with two large bristles dorsally near distal-prolateral edge, three shorter translucent hairs (several more of them on movable finger) and a brush of fine hairs prolaterally. Fixed finger with two ventral and four dorsal teeth; medial and basal dorsal tooth fused to form a bicuspid. Movable finger with five dorsal teeth, the two basal ones being the smallest; ventrally with three teeth, i.e. one distal and two medial ones.

*Pedipalp* (Figs 15-23): Trichobothrial pattern constant, corresponding to type A of Vachon (1974). Femur a little shorter than patella, with 11 trichobothria, d2 (nomenclature of trichobothria following Sissom, 1990) short and translucent (Fig. 17). Patella with two distinct tooth-like tubercles ventro-proximally and with 13 trichobothria, d2 short and translucent (Figs 18-19). Chela slender, males with a weak lobe on movable finger (not illustrated). 15 trichobothria present; esb, Esb and Eb3 short and translucent (Figs 20-21). Teeth on inner side of fingers arranged in a characteristic pattern, interspersed with short, stiff bristles, i.e. groups of two retrolateral and one prolateral bristle and several of them at the tip (Figs 22-23).

*Legs* (Figs 24-28): increasing in length from leg 1 - 4. All legs with a pair of ventral pedal spurs distally on basitarsus. Proventral spur carrying a hair and a translucent tooth originating proximally on it (Fig. 28). Retroventral spur with a small tooth close to spur (Fig. 27). Legs 3 and 4 with a tibial spur distally (Fig. 26). Dorsal distitarsus with a conspicuous distal hair on an elevated base (Fig. 28). Apotele with three claws (Fig. 27).

**Remarks:** *Mesobuthus cyprius* sp. n. differs from most populations of *M. gibbosus* by its pigmentation pattern on the mesosomal tergites, particularly by the width of the pale interspace between the median and the paramedian dark stripe which is less than 1-1.5 times the width of the latter at the posterior margin of tergites IV-VI. In most populations of *M. gibbosus*, the width of this interspace is at least more than 2 times the width of the paramedian stripe which may even be absent. However, this



FIGS 7-12

*Mesobuthus cyprius* sp. n. – 7. Left hemispermatophore, total view; surrounding tissue of paraxial organ removed, except in distal part of flagellum. – 8, 9. Lobes at truncal flexure. – 10. Metasoma segments 4, 5, and telson of male, dorsal view. – 11. Ditto, lateral view. – 12. Ditto, ventral view.

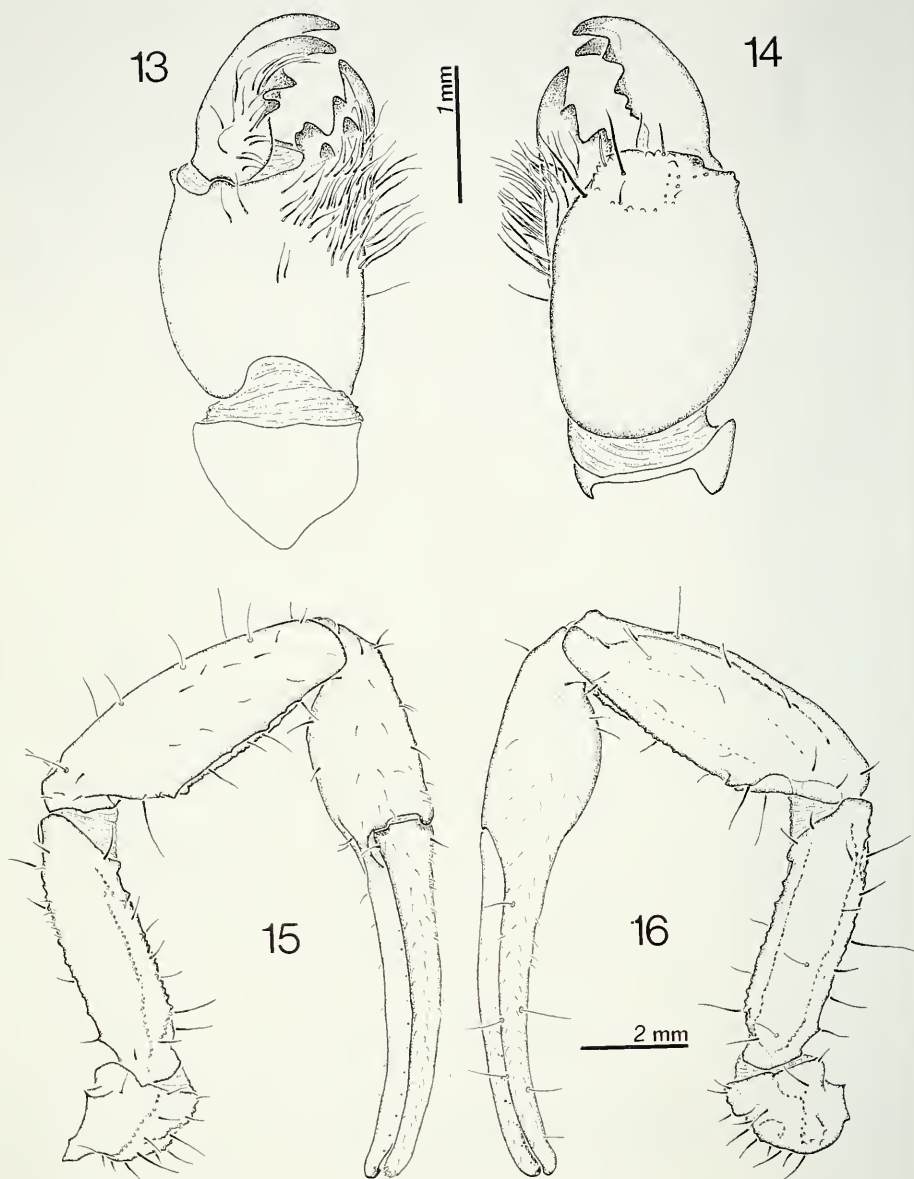
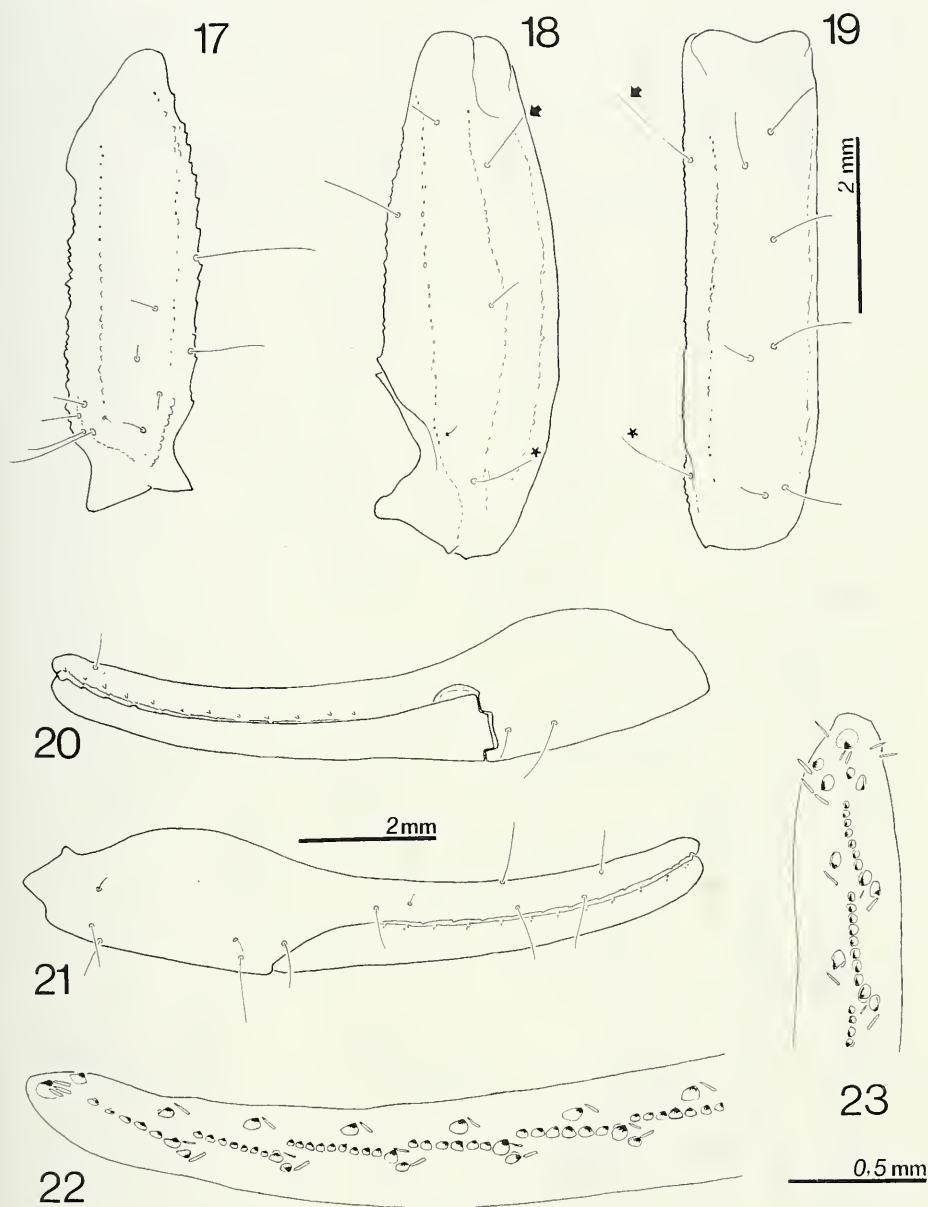


FIG. 13-16

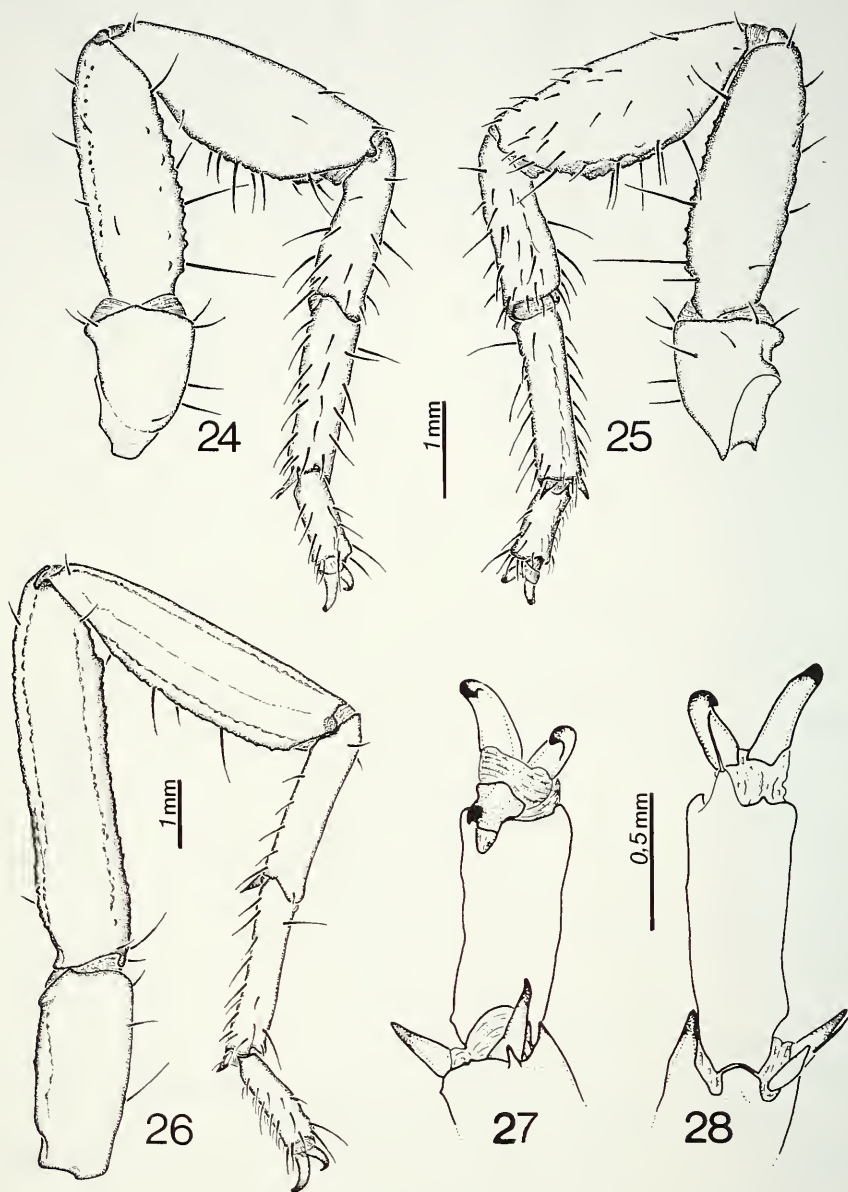
*Mesobuthus cyprius* sp. n., male – 13. Right chelicera, ventral view; prolateral hair brush simplified. – 14. Ditto, dorsal view. – 15. Right pedipalp, ventral view. – 16. Ditto, dorsal view.



FIGS 17-23

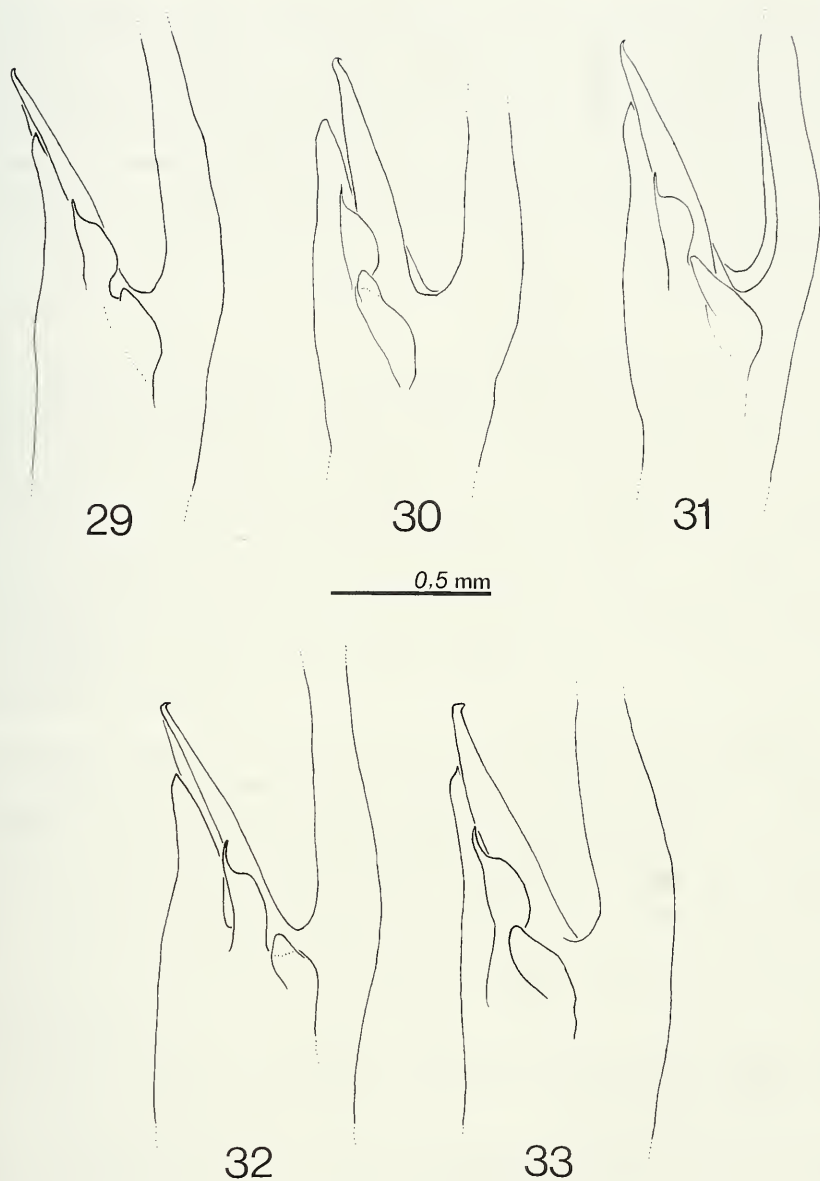
*Mesobuthus cyprius* sp. n., male – 17. Right palpal femur, trichobothrial pattern, dorsal view. – 18. Right palpal patella, trichobothrial pattern, dorsal view. – 19. Ditto, retrolateral view; arrow and asterisk in Figs 18, 19 indicate the same trichobothria. – 20. Right chela, trichobothrial pattern, proventral view. – 21. Ditto, retrodorsal view. – 22. Distal part of fixed finger of right chela, inner side. – 23. Tip of movable finger of right chela, inner side.





FIGS 24-28

*Mesobuthus cyprius* sp. n., male legs. - 24. Left leg I, prolateral view. - 25. Ditto, retrolateral view. - 26. Left leg IV, prolateral view. - 27. Left tarsus I, ventral view: hairs omitted. - 28. Ditto, dorsal view.



FIGS 29-33

*Mesobuthus gibbosus*, lobes at truncal flexure of left hemispermatophore in specimens from different regions; same magnification as in Figs 8-9. - 29. Euboea (GR). - 30. Rhodes, Kolympia (GR). - 31. Rhodes, Monolithos (GR). - 32, 33. Selale (TR).

distinction is not reliable. On the one hand the mesosomal pattern is hardly visible in some animals, on the other hand specimens of *M. gibbosus* from the Peloponnesian peninsula (Mathia) and from Crete can also show this pigmentation pattern. The Peloponnesian individuals of *M. gibbosus* differ by a characteristic row of bristles on the mesosomal tergites, which cannot be found in *Mesobuthus cyprius* sp. n. There, only some scattered bristles, if any at all, do occur. Some of the Cretean specimens of *M. gibbosus* examined cannot be distinguished from *M. cyprius* sp. n. by means of external morphology, because they possess a similar pigmentation pattern and lack mesosomal bristle rows.

## DISCUSSION

The allozyme data generally revealed a low genetic variability (Table 1), as already observed in earlier studies on scorpions of the family Euscorpiidae (Stockwell 1992; Gantenbein *et al.*, 1998, 1999). One exception is the population of *M. gibbosus* from the island of Rhodes which shows a higher level of heterozygosity as compared to the other *Mesobuthus* populations. This is mainly due to three gene loci (Ark, Gtdh, Sod) (Table 1). Specimens of *M. gibbosus* from Rhodes that are heterozygous at these three loci possess one allele, which is elsewhere only found in the samples from the Peloponnesus, and another allele, which is common in the Cretean and Anatolian populations. We speculate that this pattern indicates hybridization between western Aegean populations and eastern Aegean populations on the island of Rhodes. This hybridization may have resulted from secondary contact between the autochthonous Rhodes population and an introduced population of Greek origin.

The allozyme gene frequencies indicate that there are different population groups within *Mesobuthus*. In the NJ and ML tree, the Cyprus clade is separated from the other clades by a long branch (Figs 2-3). Both approaches clearly underline that the Cyprus scorpions have diverged considerably. Since the Biological Species Concept (BSC) (Mayr 1942) can hardly be applied to allopatric populations, it remains unsolved if the Cyprus population represents a separate species in this sense. Consequently, there are no objective arguments for the taxonomic rank of this population. However, our expectation that this island population remained isolated from the mainland populations since the colonisation 5.2 Myrs ago is supported by a high genetic divergence of the Cyprus clade and by the noninterchangeable morphology of the hemispermatothores. Taking these two arguments into account we, therefore, designate to the Cyprus population the rank of a species.

It is remarkable that *M. cyprius* sp. n. appears genetically distinct and highly differentiated, but can hardly be characterised by means of classical morphology. Such 'hidden' taxa are well known from other animal groups (e. g. mosquitos, polychaetes, myriapodes) (Narang *et al.*, 1989), especially on islands. The only characters that can be used for a morphological distinction are found in the hemispermatothores, in the mesosomal pigmentation pattern and in a lack of mesosomal bristle rows. The last character is a negative trait and presumably represents a plesiomorphic state as compared to most populations of *M. gibbosus*. The bristle row was found in specimens

of *M. gibbosus* from the Peloponnesus (Mathia) and from Rhodes (Petaloudes). The pigmentation pattern seems to be quite useful, because we found only limited variation there (but compare paragraph 'remarks' above). The taxonomic value of the pigmentation pattern was underlined by Lourenço (1983), who claimed that species with polymorphic colouration or pigmentation are an exception among buthids. As a conclusion, only a single reliable distinctive morphological character of *M. cyprius* sp. n. remains discriminant, i.e. the shape of the basal lobes of the hemispermatophores.

Based on a single male, Schenkel (1947) described a new variety of *M. gibbosus* from central Anatolia (collected along the road between Sivas and Amasya, Turkey). Kinzelbach (1975) elevated Schenkel's variety to subspecies rank. He distinguished *M. g. gibbosus* (Brullé, 1832) in the south of the Balkan Peninsula, Northern Sporades, Cyclades, from *M. g. anatolicus* (Schenkel 1947) living in Crete, Anatolia, and Cyprus, using differences in the number of pectinal teeth as the main character. Furthermore, he argued that these two subspecies were separated since the Miocene (12-25 Myrs BP). Kritscher (1993) refuted Kinzelbach's arguments for a separate subspecies *anatolicus* and rejected this taxon. The allozyme data now partially support the existence of two subspecies in Kinzelbach's sense. The Greek mainland populations are genetically slightly distinct from the Crete-Anatolian population group (Figs 2, 3). However, the Cyprus population (= *Mesobuthus cyprius* sp. n.) is genetically considerably more differentiated from all others, as discussed above. This fact contradicts the existence of *M. g. anatolicus* on Cyprus as proposed by Kinzelbach.

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